Syndromes and Disorders Associated with Omphalocele (I): Beckwith–Wiedemann Syndrome

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SUMMARY

Beckwith–Wiedemann syndrome (BWS, OMIM 130650) is characterized by macrosomia, macroglossia, visceromegaly, hemihypertrophy, abdominal wall defects, ear creases/pits, neonatal hypoglycemia, polyhydramnios, placentomegaly, placental mesenchymal dysplasia, cardiac defects, nevus flammeus, hemangiomata, and an increased frequency of embryonal tumors. This article provides an overview of BWS including the genetics, genetic diagnosis, genotype/epigenotype–phenotype correlations, association with assisted reproductive technology, and prenatal diagnosis. Omphalocele is an important sonographic marker for BWS. Prenatal detection of omphalocele, fetal overgrowth, polyhydramnios, increased abdominal circumference, placentomegaly and/or placental mesenchymal dysplasia should alert one to the possibility of BWS and prompt a genetic investigation and counseling for BWS. [*Taiwan J Obstet Gynecol* 2007;46(2):96–102]

Key Words: Beckwith–Wiedemann syndrome, genetics, omphalocele

Introduction

Beckwith–Wiedemann syndrome (BWS; OMIM 130650) is characterized by macrosomia, macroglossia, visceromegaly, hemihypertrophy, abdominal wall defects, ear creases/pits, neonatal hypoglycemia, adrenocortical cytomegaly, dysplasia of the renal medulla, and an increased frequency of adrenal carcinoma, nephroblastoma, hepatoblastoma and rhabdomyosarcoma. Other associated abnormalities include polyhydramnios, placentomegaly, placental mesenchymal dysplasia, cardiomegaly, structural cardiac anomalies, nevus flammeus, hemangiomata, advanced bone age, and midfacial hypoplasia. BWS is the most common overgrowth syndrome. Elliott et al reported the following in 74 pediatric patients with BWS: macroglossia (97%); pre- or postnatal gigantism (88%); abdominal wall defects such as omphalocele, umbilical hernia or diastasis recti (80%); ear creases or posterior helical ear pits (76%); hypoglycemia (63%); facial nevus flammeus (62%); renal anomalies such as nephromegaly, multiple calyceal cysts or hydronephrosis (59%); hemihypertrophy (24%); congenital cardiac malformations (6.5%); intestinal malrotation (5%); neoplasia (4%); moderate/severe mental retardation (4%); polydactyly (3%); and cleft palate (2.5%) [1]. The mode of inheritance is complex and the patterns include autosomal dominance with variable expressivity, contiguous gene duplication at 11p15, genomic imprinting resulting from a defective or absent copy of the maternally derived gene at 11p15, and mutations of the gene in the region of chromosomes 11p15 and 5q35.

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Genetics of BWS

Genes associated with BWS at the chromosome region of 11p15 are organized into two distinct

domains: domain 1 and domain 2. Domains 1 and 2 are respectively controlled by two imprinting centers of parent-of-origin-specific differentially methylated regions (DMRs), DMR1 and DMR2 [2,3]. Domain 1, which is located at the distal end of 11p15, contains the imprinted genes of insulin-like growth factor II (*IGF2*) and *H19*, and the imprinting center DMR1 (or imprinting center 1, IC1). Domain 2, which is centromeric to domain 1, contains several imprinted genes including *CDKN1C* (also known as cyclin-dependent kinase inhibitor 1C), *KCNQ1* (also known as *KvLQT1* or potassium channel, voltage-gated, KQT-like subfamily member 1), *KCNQ1OT1* (also known as *LIT1*, *KCNQ1*-overlapping transcript 1 or long QT intronic transcript 1), *PHLDA2*, *SLC22A18*, and the imprinting center DMR2 (or imprinting center 2, IC2). *PHLDA2* and *SLC22A18* are not directly implicated in BWS [3].

Domain 1

(1) IGF2

IGF2 (OMIM 147470) encodes insulin-growth factor II, which is an embryonic growth factor and a regulator of cell proliferation. The functions of this gene include mediation of growth hormone action, stimulation of growth of cultured cells, stimulation of the action of insulin, and involvement in development and growth. *IGF2* is a paternally expressed and maternally imprinted gene. Disruption of *IGF2* imprinting, and paternal duplication of 11p15 or paternal uniparental disomy 11 (UPD11) will cause increased expression of *IGF2* and BWS.

(2) H19

H19 (OMIM 103280) encodes a biologically active, nontranslated mRNA that may function as a tumor suppressor [4]. *H19* is a maternally expressed and paternally imprinted gene. The maternal *H19* promoter is unmethylated, and the paternal *H19* promoter is methylated. Gain of methylation at maternal *H19* promoter will cause loss of *H19* expression and expression of biallelic *IGF2* and BWS.

(3) DMR1

DMR1 is located several kilobases upstream of *H19* and is an imprinting center. The activity of DMR1 is dependent on the vertebrate enhancer-blocking protein, CTCF (OMIM 604167) [5]. The CTCF-dependent enhancer-blocking element acts as an insulator. Normally, the maternal DMR1 is unmethylated, permitting the binding of CTCF to DMR1, thereby blocking the access of the *IGF2* promoter to the downstream enhancer. On the other hand, the paternal DMR1 and *H19* promoter are methylated, thus silencing the *H19* promoter and preventing the binding of CTCF to DMR1.

Domain 2

(1) KCNQ1

KCNQ1 (OMIM 607542) encodes a protein with structural features of a voltage-gated potassium channel. Mutations of *KCNQ1* are associated with long QT syndrome. *KCNQ1* is maternally expressed in most tissues except the heart [6].

(2) DMR2

DMR2 (also known as *KvDMR1* or *KvLQT1* DMR) is located within intron 10 of *KCNQ1* and is an imprinting center. Smilinich et al identified DMR2 in the maternally methylated CpG island within intron 10 of *KCNQ1* and proposed that hypomethylation at maternal DMR2 represents a distinct epigenetic anomaly associated with biallelic expression of *IGF2* and BWS [7]. Normally, the paternal DMR2 is unmethylated, allowing the expression of *KCNQ1OT1* and silencing of *CDKN1C*; the maternal DMR2 is methylated, thus causing *KCNQ1OT1* to be silenced and *CDKN1C* to be expressed. Loss of methylation at maternal DMR2 occurs in 50% of BWS cases [2,7–10].

(3) KCNQ1OT1

KCNQ1OT1 (OMIM 604115) is a noncoding RNA with antisense transcription to *KCNQ1*. The 5' end of the transcript overlaps with DMR2. *KCNQ1OT1* is normally expressed from the paternal allele and methylated on the maternal allele.

(4) CDKN1C

CDKN1C (OMIM 600856) encodes the p57(KIP2) protein which is a potent tight-binding inhibitor of several G1 cyclin/cyclin-dependent kinase complexes and a negative regulator of cell proliferation [11]. *CDKN1C* is a tumor suppressor gene and a negative regulator of fetal growth. This gene is maternally expressed and is regulated by DMR2. Loss of methylation at maternal DMR2 is associated with decreased expression of *CDKN1C* [3]. Mutations in *CDKN1C* are associated with 40% of familial BWS [12–14].

NSD1

Mutations in *NSD1* are associated with Sotos syndrome (OMIM 117550), an overgrowth syndrome. There is clinical overlap between BWS and Sotos syndrome. Baujat et al detected two *NSD1* mutations in a series of 52 patients with BWS and two 11p15 anomalies in a series of 20 patients with Sotos syndrome [15].

Baujat et al suggested that *NSD1* may be involved in imprinting of the 11p15 region [15].

Genetic Diagnosis of BWS

Analysis of frequency of genetic abnormalities in patients with BWS found: loss of methylation at maternal DMR2 in 50%; paternal UPD11 in 20%; mutations in *CDKN1C* in 10%; gain of methylation at maternal DMR1 in 2–7%; 11p15 chromosome translocation/inversion or duplication in < 1%; and unknown etiology in 13–15% of the cases [3]. Genetic investigation of BWS includes cytogenetic analysis of chromosome 11p15 duplication, inversion or translocation; and molecular analysis of UPD11, loss of methylation at DMR2, gain of methylation at DMR1 or *H19*, and mutations in *CDKN1C*. Array-based SNP genotyping has been proven to be a fast, cost-effective, and reliable approach for whole genomic UPD screening and is very useful for genetic diagnosis of patUPD11p associated with BWS [16,17]. Quantitative methylation-sensitive polymerase chain reaction has been proven to be a rapid and highly quantitative test for assessment of DNA methylation at both DMR1 and DMR2 at 11p15 [18].

Genotype/Epigenotype–Phenotype Correlations in BWS

Variations in phenotypic expression of BWS have been linked to specific molecular subgroups. Engel et al found that omphalocele was highly correlated with loss of methylation at DMR2 and germline *CDKN1C* mutations but was less correlated with gain of methylation at DMR1 and UPD11p15 [19]. In their study of patients with BWS, 20 of 29 cases (69%) with DMR2 defects had omphalocele, 13 of 15 cases (86.7%) with mutations in *CDKN1C* had omphalocele, and none of the five cases with DMR1 defects or 22 cases with UPD11p15 had omphalocele. Engel et al found that embryonal tumors were confined to BWS patients with UPD11p15 and DMR1 defects, but not BWS patients with DMR2 defects [19]. Gaston et al found that BWS patients with UPD11p15 and DMR2 defects had an increased risk of tumors [9]. Weksberg et al found that BWS patients with UPD11p15 and DMR1 defects carried the highest tumor risk with preferential development of Wilms' tumor, whereas BWS patients with DMR2 defects had a lower tumor risk but were susceptible to non-Wilms' tumor [20]. Bliek et al found an increased tumor risk in BWS patients with DMR1 defects but not in patients with DMR2 defects [10]. DeBaun et al found that UPD11p15 was associated with hemihypertrophy, cancer, and hypoglycemia and that DMR2 defects were highly associated with omphalocele and macrosomia [21]. In a study of 200 patients with BWS (16 with *CDKN1C* mutations, 116 with DMR2 defects, 14 with DMR1 defects, and 54 with UPD11p15), Cooper et al found that (1) hemihypertrophy was strongly associated with UPD11p15; (2) omphalocele was associated with DMR2 defects or *CDKN1C* mutations but not UPD11p15 or DMR1 defects; (3) macrosomia was significantly higher in cases with *CDKN1C* mutations or DMR2 defects; and (4) the tumor risk was significantly higher in cases with UPD11p15 or DMR1 defects than in cases with DMR2 defects or *CDKN1C* mutations [22]. Smith et al concluded that (1) UPD11p15 is strongly associated with hemihypertrophy and tumors; (2) DMR1 defects have a higher cancer risk than DMR2 defects and *CDKN1C* mutations; (3) omphalocele is highly associated with DMR2 defects or *CDKN1C* mutations; (4) ear pits/creases are most prevalent in *CDKN1C* mutations and DMR2 defects; and (5) cleft palate occurs only in *CDKN1C* mutations [23]. UPD11p15, DMR1 defects, and DMR2 defects have all been found in male monozygotic twins with BWS [23]. Only DMR2 defects, however, have been found in female monozygotic twins with BWS [24]. This suggests that the developmental processes (such as X inactivation and the developmental time lag for female embryos in the preimplantation phase in monozygotic twinning) limited to females rather than males cause an increased rate of epigenetic errors at DMR2 [23–25].

BWS and Assisted Reproductive Technology

DeBaun et al [26], Gicquel et al [27], and Maher et al [28] suggested that *in vitro* fertilization (IVF) and embryo transfer, and intracytoplasmic sperm injection procedures might cause epigenetic and imprinting alterations at the centromeric imprinted 11p15 locus and increase the risk of BWS. Maher et al reported that 4% (6/169) of the patients with BWS were conceived by assisted reproductive technology (ART) versus the background rate of 0.997% ART births from the general population in the United Kingdom, giving a threefold increase in prevalence of ART in patients with BWS [28]. DeBaun et al reported that 4.6% (3/65) of the patients with BWS were conceived by ART versus the background rate of 0.8% in the United States, giving a sixfold increase in the rate of ART in children with BWS [26]. Gicquel et al reported that 4% (6/149) of the patients with BWS were conceived by ART versus

the background rate of 1.3% in the general population in France, giving a threefold increase in the rate of ART in children with BWS [27]. The data reported by DeBaun et al [26], Gicquel et al [27], and Maher et al [28] suggest that approximately 4% of individuals with BWS are conceived using ART. Molecular studies have shown an association between IVF and BWS related to hypomethylation at maternal DMR2 [26–31]. Halliday et al, in a case-control study, found 37 cases of BWS among 1,316,500 live births in Victoria, Australia between 1983 and 2003, giving an overall BWS prevalence of 1/35,580 live births for this period [29]. Of the 37 with BWS, four were conceived by IVF, and during this period there were 14,894 babies born after IVF, giving an absolute risk of 4/14,894 of having a liveborn baby with BWS conceived by IVF. Halliday et al suggested that the overall risk of BWS in the population of children conceived by IVF is about 1/4,000 or nine times greater than in the general population [29]. Chang et al studied the association between BWS and ART in a series of 19 patients with BWS and concluded that no specific ART method, specific *in vitro* media, or timing of embryo is associated with BWS [32]. However, Menezo et al pointed out that the impact of methionine in the culture media on methylation should be considered in epigenetic changes [33]. Menezo et al suggested that DNA hypomethylation and the epigenetic problems are controlled by S-adenosylmethionine produced by S-adenosylmethionine synthetase, which is active in the mouse and human oocytes, and early pre-implantation embryo and requires methionine for its synthesis [33,34]. It is hypothesized that the methionine content in the commercial media used in ART is the factor affecting DNA methylation and imprinting [33,35,36]. Moreover, ovarian stimulation may increase the risk of imprinting disorders [32,33,37]. Sutcliffe et al, in a British survey of ART and imprinting disorders, found a significantly increased frequency of ART in children with BWS (2.9%; 95% confidence interval, 1.4–6.3% *vs.* 0.8% expected) [31]. Rossignol et al further noted that epigenetic defect of patients with BWS born after ART was not restricted to the 11p15 region [30]. They found that three of 11 (27%) patients conceived using ART displayed an abnormal methylation at a locus other than *KCNQ1OT1* [30]. In their study, two patients with BWS showed demethylation of *IGF2R* DMR2 at 6q26, and one patient with BWS showed demethylation of *SNRPN* at 15q11–13 in addition to demethylation of *KCNQ1OT1*. Chen et al reported the prenatal identification of fetal overgrowth and omphalocele, and lumbosacral myelomeningocele in two ART pregnancies, respectively and suggested a careful investigation of birth defects in pregnancies achieved by ART, including a sonographic screening of fetal overgrowth, abdominal wall defects and neural tube defects, and a molecular survey of human imprinting disorders [38].

Prenatal Diagnosis of BWS

Prenatal identification of BWS is helpful for perinatal counseling and perinatal management, such as the mode of delivery, pediatric care for neonatal hypoglycemia, airway obstruction, respiratory distress and congestive heart failure, the risk of malignancy, and genetic testing of family members. Prenatal diagnosis of BWS is associated with abnormal sonographic findings, a positive family history, and abnormal cytogenetic and/or molecular results. Based on a clinical review of 19 prenatally detected BWS, Williams et al suggested that prenatal diagnosis of BWS can be reliably made by either two major criteria or one major criterion plus two minor criteria [39]. The major criteria include macroglossia, macrosomia (> 90th percentile), and an abdominal wall defect. The minor criteria include aneuploidy/abnormal loci, polyhydramnios, nephromegaly, and renal dysgenesis/dysplasia and adrenal cytomegaly confirmed by pathologic diagnosis. Patients with BWS are at risk for developing malignant tumors (such as Wilms' tumor, adrenocortical carcinoma, hepatoblastoma, hepatocellular carcinoma, glioblastoma, neuroblastoma, rhabdomyosarcoma, malignant lymphoma, pancreatoblastoma, carcinoid tumors, congenital mesoblastic nephroma, renal cell carcinoma, myelodysplasia, yolk sac tumor, and intratubular germ cell neoplasm) and benign tumors (such as adrenal adenoma, teratoma, fibroadenoma, fibrous hamartoma, ganglioneuroma, myxoma, cardiac hamartoma, chorangioma, digital fibroma, hepatic hemangioma, bladder neck polyp, and bladder hamartoma) [40]. Wiedemann reported that the overall risk for tumor development in children with BWS was 7.5% [41]. DeBaun et al reported that the average annual incidence of cancer in patients with BWS in the first 4 years of life was 0.027 cancer per person-year [42]. The relative risk of cancer was 816 for Wilms' tumor, 197 for neuroblastoma, and 2,280 for hepatoblastoma. Hemihypertrophy was the only clinical feature associated with a significantly increased relative risk of cancer. Congenital cysts or tumors in the adrenal gland or pancreas associated with BWS have been observed by prenatal ultrasound. Merrot et al reported the prenatal detection of a right hemorrhagic adrenal cyst at 21 gestational weeks in a fetus with incomplete BWS [43]. Gocmen et al reported the prenatal sonographic findings of bilateral hemorrhagic adrenal cysts at 33 gestational weeks in a fetus with BWS [44]. Izbizky et al reported the prenatal

sonographic findings of bilateral adrenal carcinoma, polyhydramnios, macrosomia, and placentomegaly at 26 gestational weeks in a fetus with incomplete BWS [45]. Fremond et al reported the prenatal detection of a congenital pancreatic cyst at 24 gestational weeks in a fetus with omphalocele and BWS [46]. Pelizzo et al reported the prenatal sonographic findings of congenital pancreatoblastoma at 20 gestational weeks in a fetus with BWS [47]. Visceromegaly, placentomegaly, and placental mesenchymal dysplasia are associated with BWS and can be detected prenatally by ultrasound. Mulik et al reported the prenatal sonographic findings of gross hepatomegaly, an enlarged pancreas, and placental mesenchymal dysplasia in a fetus with BWS [48]. Drut and Drut reported the findings of nonimmune fetal hydrops and placentomegaly in familial BWS with trisomy 11p15 [49]. Lage reported three cases of fetal omphaloceles (one had diagnostic BWS and two had pathologic features suggestive of BWS) with placentomegaly, massive hydrops of placental stem villi, and diploid DNA content [50]. McCowan and Becroft reported the findings of placentomegaly, omphalocele, and cystic hydrops of stem villi in a pregnancy with fetal BWS [51]. Hillstrom et al reported the sonographic findings of placental villous hydrops and omphalocele associated with fetal BWS [52]. Placental mesenchymal dysplasia is characterized by an enlarged hydropic placenta with numerous cyst-like villi mimicking partial mole, histologic features of enlarged stem villi with cistern formation, and a lack of trophoblastic hyperplasia [53–57]. Cohen et al suggested that multiple cystic changes in the placenta (by prenatal ultrasound), a normal or slightly increased level of maternal serum β-human chorionic gonadotrophin and an elevated level of maternal serum α-fetoprotein (with maternal serum screening), and the presence of a diploid fetus are indicative of placental mesenchymal dysplasia [58]. Placental mesenchymal dysplasia can be a characteristic prenatal sonographic feature of BWS [50–52,56,59,60]. Placental mesenchymal dysplasia is associated with BWS, intrauterine growth restriction, and fetal demise in the majority of the cases but can also be associated with normal fetuses [57,58,61]. Cohen et al reviewed 66 reported cases with placental mesenchymal dysplasia and found that 15 cases (23%) were associated with BWS [58]. Pregnancies with BWS fetuses, placentomegaly and placental mesenchymal dysplasia may present maternal hypertension and proteinuria [51]. McCowan and Becroft suggested that gestational proteinuric hypertension in association with ultrasound findings of placentomegaly with or without cystic changes in the placenta should be considered in the diagnosis of BWS [51]. Reish et al suggested that prenatal detection of fetal overgrowth, polyhydramnios, increased abdominal circumference, omphalocele, and placentomegaly should alert one to the possibility of BWS and prompt the molecular and cytogenetic analysis of BWS [62]. Grati et al reported the detection of a paternal segmental UPD11 by molecular investigation of amniotic fluid cell cultures in two fetuses with apparently isolated omphalocele and suggested that the necessity of molecular analysis in all cases with fetal omphalocele [63].

Conclusion

This article provides an overview of BWS including the genetics, genetic diagnosis, genotype/epigenotype–phenotype correlations, association with ART, and prenatal diagnosis. Omphalocele is an important sonographic marker for BWS. Prenatal detection of omphalocele, fetal overgrowth, polyhydramnios, increased abdominal circumference, placentomegaly and/or placental mesenchymal dysplasia should alert one to the possibility of BWS and prompt the genetic investigation and counseling for BWS.

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